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VULVOVAGINAL CANDIDIASIS AMONG FEMALES OF REPRODUCTIVE AGE AT A TERTIARY CARE CENTER, WESTERN UTTAR PRADESH

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ABSTRACT: Background: Vulvovaginitis is an inflammation or infection of vulva and vagina. *Candida* is a commensal micro-organism which mainly colonizes the mucosal surfaces of human body such as gastrointestinal tract, respiratory tract and genitourinary tract. This study aimed to find prevalence of vulvovaginal candidiasis and determine antifungal susceptibility pattern of clinical *Candida* isolates among samples at U.P.U.M.S, Saifai. **Materials and Methods:** A total of 112 vaginal swabs were collected from patients with symptoms suggestive of vulvovaginal candidiasis. *Candida* species were isolated and identified using standard laboratory techniques. Antifungal susceptibility testing was performed using Broth microdilution method. **Results:** Out of 58 *Candida* isolates the most common clinical strains isolated were *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candidakrusei*, *Candida dubliniensis* and *Candida parapsilosis*. *Candida albicans* was the most prevalent species (23.2%). Mean value of MIC for itraconazole against *Candida* species ranges from 0.063-1.00µg/ml, for terbinafine against *Candida* species was found to be 0.031-4.00µg/ml, for voriconazole against *Candida* species was found to be 0.012-27.897µg/ml and for fluconazole it was 0.003-42.50µg/ml. **Conclusion:** This study demonstrates the importance of investigating the prevalence and antifungal susceptibility pattern of VVC. Our findings have implications for treatment strategies and highlight the need for continued monitoring of antifungal susceptibility patterns.

INTRODUCTION: Vulvovaginal candidiasis (VVC) is a common gynaecological disorder affecting millions of women worldwide¹. The increasing prevalence of VVC, coupled with rising antifungal resistance, poses significant therapeutic challenges¹. *Candida* species, particularly *Candida albicans*, are the primary causative agents of VVC.

However, the emergence of non-*albicans* species and antifungal resistance has complicated treatment outcomes². This study aims to bridge this knowledge gap by investigating the prevalence, species distribution, and antifungal resistance patterns of VVC in a tertiary care setting.

Understanding the local epidemiology and antifungal susceptibility patterns is crucial for developing effective treatment strategies and improving patient outcomes³. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-S4 micro broth dilution method. The present study aimed to determine the prevalence of

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Candida infection in patients and the *in-vitro* activity of antifungal drugs itraconazole, voriconazole, fluconazole and terbinafine⁴.

MATERIALS AND METHOD: This prospective cross-sectional study was conducted in the Mycology Laboratory, Department of Microbiology, Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah, Uttar Pradesh (India) from January 2023 to July 2024.

The Institutional Ethics Committee approved the study (Clearance code: 66/2022-23). A total of 112 high vaginal swabs were collected with symptoms like white curdy discharge, itching, vulvar edema, abdominal pain, foul smelling *etc.*, were included in this study.

Isolation: During the visit, the women were examined in lithotomy position, a clean bi-valve speculum was inserted into vagina. Once vagina is inspected properly, two high vaginal swab clinical samples (vaginal discharge) were collected. After immediate transportation, the swabs were processed in the microbiology laboratory. First swab was used for preparing wet mount and Gram staining, for fungal identification. The other swab was used for fungal culture. The specimen was inoculated onto Sabouraud's dextrose agar (SDA) supplemented with antibacterial antibiotics then aerobically incubated at 37°C for 24-48 h and well isolated colonies were used to plate on Hi-Chrom agar. The LPCB mount was prepared from the colonies to examine yeast cells and pseudo hyphae.

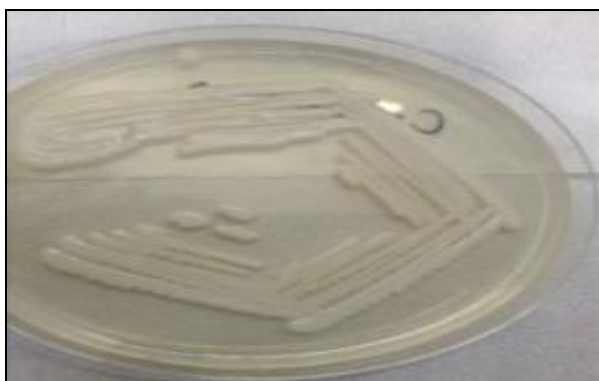


FIG. 1: APPEARANCE OF CANDIDA COLONIES ON SDA AGAR

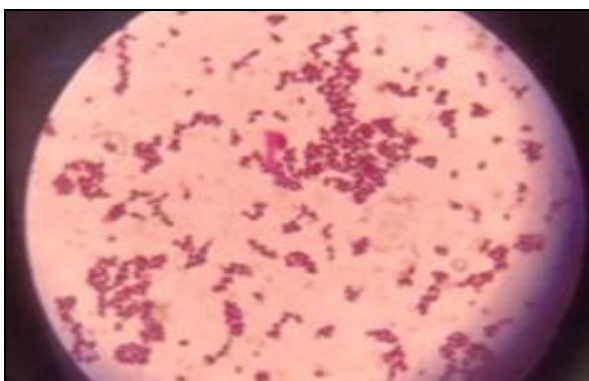


FIG. 2: GRAM'S STAINING SHOWING BUDDING YEAST CELLS AT 100X (OIL IMMERSION)

Identification: Any visible growth seen on the SDA slope was processed for identification of the species. From an isolated colony, macroscopic examination, Gram staining, germ tube test, and chlamydospores formation. The yeast like, pasty, and creamy colonies that showed Gram-positive budding yeast cells with pseudohyphae on microscopic examination and negative urea

hydrolysis test were further processed for Candida speciation on CHROM agar. When the growth of yeast colonies was observed, the Gram stain method was used to verify the absence of bacterial contamination. The yeasts were identified via carbohydrate assimilation test and by using the Vitek 2 System (BioMerieux I'Etoile, France) according to the manufacturer's instructions.

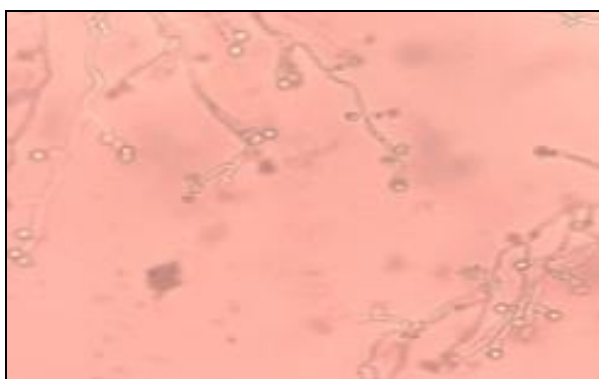


FIG. 3: HICROME CANDIDA DIFFERENTIAL AGAR SHOWING DIFFERENT SPECIES OF CANDIDA



FIG. 4: PSEUDOHYPHAE ON KOH MOUNT

Antifungal Susceptibility Testing: *In-vitro*, antifungal susceptibility testing was performed, and minimum inhibitory concentration (MICs) was determined according to the recommendations stated in the Clinical and Laboratory Standards Institute M27-S4 documents. Voriconazole, terbinafine, fluconazole and itraconazole were used for preparation of the CLSI microdilution trays. The antifungal agents were diluted in the standard

RPMI-1640 medium (Sigma Chemical Co.) buffered to pH 7.0 with 0.165 M morpholine propanesulfonic acid (MOPS) (Sigma) with L-glutamine without bicarbonate to yield two times concentrations and dispensed into 96-well microdilution trays at a final concentration range of 0.472-2.134µg/ml for itraconazole, voriconazole, fluconazole and terbinafine. MIC values were determined visually after 24 h-72 h at 35°C⁵.



FIG. 5: SHOWS THE RESULT OF MICROBROTH DILUTION

RESULTS: A total of 112 vaginal samples were tested. Majority of cases fall in age group of 26-30 year i.e 33% cases. Majority of females belongs to rural population i.e. 87 cases (77.7%) due to lack of awareness, poor education and low socio-economic status. The most common chief complaint was white curdy discharge in 63 cases (56.35%), followed by itching edema and abdominal pain.

The common isolated species was *Candida albicans* (26spp) which was confirmed by germ tube test (manual test) and Vitek 2 compact system and other non albicans candida species isolated were *Candida glabrata* (11), *Candida tropicalis* (7), *Candida krusei* (6), *Candida dubliniensis* (3) and *Candida parapsilosis* (2).

For cumulative result, for fluconazole, the mean MIC is 2.134 µg/mL with a standard deviation of 5.144 µg/mL; for itraconazole, the mean MIC is 0.438 µg/mL with a standard deviation of 0.617 µg/mL; for voriconazole, the mean MIC is 0.261

µg/mL with a standard deviation of 0.376 µg/mL; and for terbinafine, the mean MIC is 0.472 µg/mL with a standard deviation of 0.839 µg/mL, though the MIC for terbinafine is based on my observation as there is no defined MIC for this antifungal.

TABLE 1: GRAM'S STAINING FINDING

Gram's stain finding	Frequency (N=112)	Percentage (%)
Budding yeast-like cells	58	51.8
No budding yeast-like cell	54	48.2
Total	112	100.0

TABLE 2: ISOLATED CANDIDA SPECIES

Isolated candida species	Frequency (N=58)	Percentage (%)
<i>Candida albicans</i>	26	23.2
<i>Candida dubliniensis</i>	5	4.5
<i>Candida glabrata</i>	11	9.8
<i>Candida krusei</i>	6	5.4
<i>Candida parapsilosis</i>	3	2.7
<i>Candida tropicalis</i>	7	6.3
Total	58	51.9

TABLE 3: COMPARATIVE ANALYSIS ON MIC VALUES OF ANTIFUNGAL AGENTS AMONG DIFFERENT CANDIDA SPECIES

	N	Mean	Std. Deviation	Minimum	Maximum	F-value	p-value
ITR <i>Candida albicans</i>	26	0.524	0.545	0.063	2.000	0.622	0.003*
<i>Candida dubliniensis</i>	4	0.406	0.400	0.125	1.000		
<i>Candida glabrata</i>	12	0.540	0.630	0.062	2.000		

	<i>Candida krusei</i>	6	0.573	0.472	0.063	1.000		
	<i>Candida parapsilosis</i>	3	0.708	0.505	0.125	1.000		
	<i>Candida tropicalis</i>	7	0.920	0.833	0.063	2.000		
	Total	58	0.582	0.578	0.062	2.000		
TER	<i>Candida albicans</i>	26	0.613	1.121	0.031	4.000	0.415	0.837**
	<i>Candida dubliniensis</i>	5	0.350	0.205	0.125	0.500		
	<i>Candida glabrata</i>	11	0.312	0.363	0.031	1.000		
	<i>Candida krusei</i>	6	0.219	0.222	0.031	0.500		
	<i>Candida parapsilosis</i>	3	0.750	1.083	0.125	2.000		
	<i>Candida tropicalis</i>	7	0.388	0.714	0.031	2.000		
	Total	58	0.472	0.839	0.031	4.000		
VORI	<i>Candida albicans</i>	26	0.052	0.035	0.012	0.110	62.435	0.000*
	<i>Candida dubliniensis</i>	5	0.223	0.097	0.125	0.325		
	<i>Candida glabrata</i>	11	16.775	6.429	2.678	27.897		
	<i>Candida krusei</i>	6	0.161	0.124	0.025	0.313		
	<i>Candida parapsilosis</i>	3	0.080	0.019	0.063	0.100		
	<i>Candida tropicalis</i>	7	0.069	0.035	0.025	0.110		
	Total	58	3.253	7.128	0.012	27.897		
FLU	<i>Candida albicans</i>	26	0.993	0.626	0.212	2.890	79.276	0.000*
	<i>Candida dubliniensis</i>	5	0.090	0.074	0.016	0.212		
	<i>Candida glabrata</i>	11	2.394	1.842	0.003	5.950		
	<i>Candida krusei</i>	6	30.017	10.256	18.250	42.500		
	<i>Candida parapsilosis</i>	3	2.175	1.514	0.625	3.650		
	<i>Candida tropicalis</i>	7	2.208	1.021	1.180	3.650		
	Total	58	4.391	9.371	0.003	42.500		

DISCUSSION: In this study, 112 women suffering from clinical symptoms of vaginitis were enrolled including 34 diabetic women and 78 non-diabetic women. Vaginal candidiasis is considered as common infection in 60-70% of women belonging to reproductive age group, who experienced the problem at least once during their lifetime.

In current study the prevalence of vulvovaginal candidiasis among reproductive age female of 25-40 years was 51.49% as shown in **Table 2**. Many studies have been carried out to analyze the prevalence of VVC in different parts of the world. In a study conducted by Brandolt TM *et al.* in 2017 the prevalence of vulvovaginal candidiasis was 49.3%⁶. In other study done by Ibrahim Hussien, Alqeer Aliyo *et al.* in 2024 the prevalence was found to be 26.8% which was less than the current study⁷. In the study done by Shirin Hasanvand *et al.* in 2017, the prevalence of vulvovaginal candidiasis was found to be 54.9%⁸. In the study done by Kombade, Sarika P; Abhishek, Kumar Sin 2021, the prevalence was found to be 43%. Which was lesser than the current study⁹. The higher prevalence of vulvovaginal candidiasis is due to low socio-economic status and lack of awareness. VVC is one of the common complaints faced by people women of various age groups. In our study, the prevalence of VVC was more in 26-30 years

(33%). 112 patients of the study in reproductive age group suffered from clinical symptoms of vaginitis, of which 58 women (51.78%) suffered from VVC. This is in agreement with earlier studies Deepa babin *et al.* in 2013 showed that highest frequency of VVC was found in 26-35 years (49.58%), followed by 18-25 years and least occurrence was among patients who were more than 40 years in their study¹⁰. Another study by Hasanvand *et al.* in 2017 also reported a higher incidence of VVC in 20-30 years aged women⁸.

The present study consists of 87(77.7%) females belonging to rural area and 25 (22.3%) belongs to urban population. Greater number of cases from rural can be due to the following reasons:

1. Lack of awareness (esp. about hygiene)
2. Poor education
3. Low socio-economic status

This correlate with other study done by Sushil kumar *et al.* in 2022 where rural area counts 65.51% of *Candida* cases as compare to urban area (34.48%)¹¹. In present the most common chief complaint was curdy white discharge (56.3%) second common was itching and edema in genital area (17%) and followed by pain in abdomen (9.8%), excessive curdy white discharge(9.8%)

burning sensation while passing urine (5.4%), dyspareunia(1.8%). This correlates well with other study done by Livia Custódio Pereira *et al.* in 2021, the common complaint was white discharge found in (77.5%) population followed by itching in genital area (79.2%) and burning sensation while passing urine (72.3%)¹². In other study done by Latha Ragunathan *et al.* in 2014 the common complaint was discharge (29.4%) followed by pain in abdomen (15.6%) and itching in genital area (13.3%)¹³.

The common species isolated in this study was *Candida albicans* (26) which was confirmed by manual tests (germ tube test) and Vitek 2 compact system. The other NAC isolated in this study was *Candida glabrata* (11), *Candida tropicalis* (7), *Candida krusei* (6), *Candida dubliniensis* (3) and *Candida parapsilosis* (2) as shown in **Table 2**. This is in concordance with other study done by Nahed Ghaddar *et al.* in 2019 in which the *C. glabrata* was the most frequently isolated species (44.5%) followed by *C. albicans* (43.4%) and *C. krusei* (12.1%)¹⁴. In the other study done by N. Song, S. Kan *et al.* in 2021 *C. albicans* (84.7%) was the most frequent, followed by *C. glabrata* (8.7%)¹⁵. In the study done by Adane Bitew *et al.* in 2018 the most common isolated species was *Candida albicans* (58.6%) followed by *Candida krusei* (17.2%), *Candida dubliniensis* (9.2%), *Candida glabrata* (3.4%), *Candida tropicalis* (2.3%) and *Candida parapsilosis* (2.3%)¹⁶.

In this study, the antifungal susceptibility testing for the clinical strains was carried out by microbroth dilution method. It was observed that MIC of itraconazole against candida species ranges from 0.063-1.00µg/ml, for terbinafine against *Candida* species was found to be 0.031-4.00µg/ml, for voriconazole against candida species was found to be 0.012-27.897µg/ml and for fluconazole it was 0.003-42.50µg/ml as shown in **Table 3**.

Overall, out of 58 isolates, 36 (62.1%) is sensitive to itraconazole while 22(37.9%) isolates are resistant. MIC of itraconazole for *Candida albicans* (26 isolates) ranges from 0.063-2.000µg/ml, for *Candida glabrata* it was found to be 0.062-2.000µg/ml, for *Candida tropicalis* it was 0.063-2.000µg/ml, for *Candida krusei* it was 0.063-1.000µg/ml, for *Candida dubliniensis* it was 0.125-

1.000µg/ml and for *Candida parapsilosis* it was 0.125-1.000µg/ml. Similar studies were observed by Ronaq Zaman *et al.* in 2022 in which he observed that Itraconazole was sensitive in 108(68.4%) and resistant in 36 (22.8%) of isolates¹⁷. In study done by Deepa Babin *et al.* in 2013 observed that *Candida albicans* were resistant to itraconazole (13.95%), *C. tropicalis* isolates were resistant to itraconazole (21.87%), *C. glabrata* isolates showed (40%) resistance to itraconazole, *C. krusei* isolates were (42%) resistant to itraconazole¹⁰.

Sensitivity of voriconazole to isolates is found to be 43(74.1%) while resistant to 15 isolates (25.9%). MIC of itraconazole for *Candida albicans* ranges from 0.012-0.110µg/ml, for *Candida glabrata* it was 2.678-27.897µg/ml, for *Candida tropicalis* it was 0.025-0.110µg/ml, for *Candida krusei* it was 0.025-0.313µg/ml, for *Candida dubliniensis* it was 0.125-0.325µg/ml and for *Candida parapsilosis* it was found to be 0.063-0.100µg/ml. Similar studies were observed by Ronaq Zaman *et al.* in 2022 in which he observed that voriconazole was sensitive in 68 (43%), SDD in 5 (3.2%) and resistant in 85 (53.8%) of isolates¹⁷. In study done by Deepa Babin *et al.* in 2013 observed that *Candida albicans* were resistant to 9.30% voriconazole, *C. tropicalis* isolates were resistant to voriconazole (18.75%), *C. glabrata* isolates showed (28%) resistance to voriconazole, *C. krusei* isolates showed (21%) resistance to voriconazole¹⁰.

For fluconazole 31(53.4%) are sensitive while 27(46.6%) are resistant. *C. krusei* is intrinsically resistant to fluconazole. The MIC for *Candida albicans* ranges from 0.212-2.890µg/ml, for *Candida glabrata* it was 0.003-5.950µg/ml, for *Candida tropicalis* it was 1.180-3.650µg/ml, for *Candida krusei* it was 18.250-42.500µg/ml, for *Candida dubliniensis* it was 0.016-0.212µg/ml, for *Candida parapsilosis* it was 0.625-3.650µg/ml. Similar studies were observed by Ronaq Zaman *et al.* in 2022 in which he observed that Fluconazole was sensitive in 39 (24.7%) and resistant in 114 (72.2%) of isolates¹⁷. In study done by Deepa Babin *et al.* in 2013 observed the overall susceptibility rate for *C. albicans* were 76%, and resistance were detected in 16.27%, *C. tropicalis* isolates were resistant to fluconazole was 25%, *C. glabrata* isolates showed (48%) resistance to

fluconazole¹⁰. For terbinafine, overall, MIC ranges from 0.031-4.000µg/ml. MIC of terbinafine for *Candida albicans* ranges from 0.031-4.000µg/ml, for *Candida glabrata* it was 0.031-1.000µg/ml, for *Candida tropicalis* it was 0.031-2.000µg/ml, for *Candida krusei* it was 0.031-0.500µg/ml, for *Candida dubliniensis* it was 0.125-0.500µg/ml and for *Candida parapsilosis* it was 0.125-2.000µg/ml. The MIC observed is according to my observation as sufficient data is not available.

CONCLUSION: The present study conducted at U. P. U. M. S Saifai Etawah concludes that VVC is prevalent in the reproductive age group of 25-40 years women which was 51.49%. Rural women were found to be more vulnerable to infection than urban women, which could be due to their low socio-economic status, lack of awareness, illiteracy, social stigma, poor hygiene or improper treatment. The study found that the most common isolated species was *Candida albicans* which was found to be most susceptible for routine antifungals drugs like fluconazole, voriconazole etc. while the predominant NAC was *Candida glabrata* and *Candida tropicalis* which showed reduced susceptibility to the routine antifungals, because of which identification up to species level and antifungal susceptibility testing (AFST) is crucial.

Azoles like Fluconazole is one of the drug of choice for treating *Candida* diseases, but due to their use as over the counter drug with clotrimazole and women who practiced antifungal self-medication, antifungal resistance emerging as a threat that is leading to treatment failure and increase in cases of recurrent VVC.

A screening program is of utmost importance and provides a guide to clinicians to prescribe the appropriate dose of anti-fungal drug to patients with signs/symptoms of VVC, which can be beneficial to quite an extent, for the treatment of vulvovaginal candidiasis. Prompt laboratory diagnosis of VVC and AFST is essential to monitor the emerging antifungal resistance.

The estimation of the prevalence of vulvovaginal candidiasis in this area will play a significant role in the control measures against VVC. Additionally, this study offers baseline data on the frequency and pattern of antifungal susceptibility of isolates of

Candida in our area that will help clinicians to formulate treatment guideline for VVC in this area.

The susceptibility pattern of isolates shows alarming rates of resistance to fluconazole and clotrimazole, emphasizing the need for alternative treatment options and antifungal stewardship. The study's findings have important implications for clinical practice, highlighting the need for:

1. Enhanced diagnostic capabilities for accurate species identification and antifungal susceptibility testing.
2. Rational use of antifungal agents, considering local resistance patterns.
3. Development of effective treatment strategies for *non-albicans Candida* species.
4. Public awareness and education on prevention and management of VVC.

Therefore, all women should be educated about symptoms and personal hygiene to solve the problem of vulvovaginal candidiasis.

This study contributes to the understanding of VVC epidemiology and antifungal resistance patterns in the region, informing evidence-based practices to improve patient outcomes and reduce the burden of this common fungal infection.

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